REMARKS/ARGUMENTS

Claims 66-90 are pending. Claims 1-65 have been canceled. Claims 66, 67 and 78 have been amended. Support for the amendments is found throughout the specification and are depicted in the drawings. Reconsideration of the rejection is respectfully requested.

Claims 67, 69-74, 78, 79 and 83-86 were rejected under 35 USC 102(b), as being anticipated by Birkenkamp et al. The rejection apparently considers a hairpin with a mismatch to be a heteroduplex. The rejection also considers the enzymes to "correct the heteroduplex." This rejection is respectfully traversed.

The present invention concerns the synthesis of new polynucleotides with variant sequences which are different from either parent strand and which have different functional properties from either parent. The important feature is the presence of at least 2 mismatches in the heteroduplex. With only one mismatch, resolving the heteroduplex regenerates one of the original parents. With two or more mismatches, resolving the heteroduplex generates a population, which includes both parents and new sequence variants having the nucleotide of one parent at one mismatch site and a different parent at a second mismatch site. The number of possible reassortant sequence variants increases exponentially with the number of mismatches and number of different parents. See the specification Figures. By contrast, Birkenkamp et al is concerned with the repair of mutations and how nature can repair a mismatch to maintain the parent sequence and the parent sequence functionality. See the introduction, title of section 3.3 etc.

Birkenkamp et al does not produce a sequence variant as required by step C. Birkenkamp et al do not perform any reassortment. Birkenkamp et al wish only to resolve the mismatch to one of the parent sequences and thus would not motivate one to even try to make a sequence variant. The products produced by Birkenkamp et al are the original top sequence with a loop or the bottom sequence without the loop and possibly complementary sequences thereto, along with the usual degradation products and artifacts. The heteroduplex is "corrected" or to become one of the parent sequences and its complement. There is no formation of a sequence variant.

To further highlight this difference, claim 87 recites the formation of at least 4 variants other than the parent sequences, which increases the diversity of the population of parent sequences. Such a combination will not occur using the Birkenkamp et al technique. Additionally, claim 88 recites screening or selecting a sequence variant. Since Birkenkamp et al does not even make one a variant, they would hardly consider screening for it.

Additionally, Birkenkamp et al use endonuclease VII to repair and restore the phage genome to maintain phage viability as stated in the introduction, second paragraph. This is the opposite from the "in vitro method of making linear sequence variants" as recited in each of the independent claims.

In the rejection, the examiner urges "Birkenkamp et al teach that the complementarity increases, resulting in homoduplex polynucleotides and an increase in diversity of the population." This is a misinterpretation of the data. The increase in bands shown in figure 3, lane 8 represents a resolution of the loop mismatch feature into base-paired DNA. This generates something identical to one of the "parent strands". (Note that Birkenkamp et al use a model system of a self-complementary hairpin instead of a more standard heteroduplex pair of strands. From the wording in the rejection the Examiner appears to recognize these as equivalents. For the purposes of our discussion, each "parent strand" is one side of the hairpin.) The presence of a "new" band in the gel after the reaction is due to the usage of a hairpin rather than two parent strands. The "new" molecule is the result of a cleaving at the loop and a resolution to one of the homoduplex "parents". Increasing complementarity in Birkenkamp et al results in resolution of the mismatch without an increase in diversity. Other bands observed are cleavage artifacts.

Applicant's position is further supported by the explanations and conclusions made by Birkenkamp et al. Birkenkamp et al proposes that T4 Endonuclease VII is involved in maintaining the integrity of the phage genome. Its purposes are to help repair, or trigger repair, of any DNA distortions that may interfere with the lifecycle of the organism to yield the original parent sequence. This is quite different from the present invention which is an artificial in-vitro reaction intending to generate many sequence variants and to increase diversity in the population of DNA molecules where one may select new sequence variants with new functional properties. Accordingly, this rejection should be withdrawn.

Claim 68 was rejected under 35 USC 103 as being unpatentable over Birkenkamp et al. In addition to the reasons given above, the examiner contends that the particular order of adding ingredients is obvious as being arbitrary. This rejection is respectfully traversed.

The order of addition can matter. Some of the enzymes involved will degrade the DNA substrate if added at the wrong time, in the wrong concentration, reacted for too long or in the wrong combination. Once overly degraded or even degraded to single nucleotides, regenerating the full length DNA is not possible. On example can be seen in the Birkenkamp et al reference itself where in Fig. 3, different combinations of enzymes give different resulting DNA bands that are never produced using all of the reagents together. Hence, without a suitable reason, it is unreasonable to conclude that the order of addition does not matter for these type of reactions. Accordingly, this rejection should be withdrawn.

Claims 75-77 and 80 were rejected under 35 USC 103(a) as being unpatentable over Birkenkamp et al in view of Arnold et al. In addition to the teachings of Birkenkamp et al mentioned above, Arnold et al is cited to show "mismatch corrections methods" to produce a heterogeneous population of homoduplex nucleic acids. From these, the rejection concludes it obvious to use the Birkenkamp et al techniques and reagents for the method of Arnold et al. The examiner specifically notes that Arnold et al teach a heteroduplex correction method. This rejection is respectfully traversed.

As with the previous rejection, DNA repair systems of the references are not the same as diversity generating reaction of present claims. Applicants are performing different reactions in vitro, which coincidentally share some of the components used in the references for a different purpose. Arnold et al transforms a heteroduplex containing plasmid into "repair competent cells" which resolve the heteroduplex. Arnold et al does not indicate what enzymes and other cellular components are being used perform the reaction. Arnold et al must use DNA "repair competent cells" because DNA "repair-deficient cells" do not function. Applicants' do not use the same set of enzymes as are used in natural DNA repair. Arnold et al provides no indication that any other combinations of enzymes are

functional. Therefore, it would not be obvious to substitute the specific set taught by Birkenkamp et al.

All of the reactions performed by Arnold et al are done in-vivo, inside an intact cell. Arnold et al does not show that their method can be performed in-vitro. Since Arnold et al does not even indicate all of cellular components that operate to perform their method, one does not even know which ones are needed. In Arnold et al section "V. Types of Host Cells" states using cells with certain enzymes in the DNA repair system. These appear to be needed. However, these are different from the enzymes used by applicants. Therefore, Arnold et al teach away from the present invention.

Further, since the cellular complexes and subcellular compartmentalization of enzymes and the like are not present in in-vitro reaction, Arnold et al has not enabled any in-vitro reaction. Therefore, it is not obvious to combine this in-vivo any in-vitro reaction of Birkenkamp et al.

Three enzymes in Birkenkamp et al are not equivalent to the machinery of a whole cell. The T4 endonuclease VII is a phage enzyme, not a normal cellular enzyme. Its use is completely contrary to the teachings of Arnold et al, which do not even use phage enzymes. Without knowing which enzymes in a cell are responsible for the results obtained by Arnold et al, one does not know which enzymes and other components to use in an in-vitro reaction absent impermissible hindsight and has no reasonable expectation of success.

Still further, Arnold et al's target substrate and products are plasmids with a heteroduplexes or a resolved heteroduplexes. Claim 66 and 67 both recite "An in vitro method of making linear sequence variants." Plasmids are not linear.

Even combining the references, we do not know what constitutes "the heteroduplex correction" components in Arnold et al and therefore there can be no conclusion that they are equivalent to those in Birkenkamp et al, which are used for a different purpose. Accordingly, the rejection should be withdrawn.

Claims 66, 81 and 82 were rejected under 35 USC 103(a) as unpatentable over Birkenkamp et al in view of Arnold et al in further view of Oleykowski et al. In addition to the teachings mentioned above, the examiner urges Oleykowski et al teaches CEL I. The

examiner concludes it obvious to use CEL I in the mismatch repair method of Birkenkamp et al. This rejection is respectfully traversed.

Oleykowski et al do not compensate for the deficiencies in Birkenkamp et al and Arnold et al. Oleykowski et al detects mutations; they do not suggest making a sequence variant or increase the diversity of the population of DNA molecules. Applicants do not want to detect mutations but rather produce something similar to them by introducing sequence changes into the population of DNA molecules. Substituting CEL I for Endonuclease VII is not merely the substitution of equivalents. CEL I operates differently by nicking only one strand after the mismatch whereas Endonuclease VII can cause a second cut on the opposite strand. Page 4602 column 1 states, "The principle of mismatch recognition by CEL I appears to be different from T4 endonuclease VII, which has also been used for enzyme mutation detection (11,12). The later is a resolvase..." Therefore, these two enzymes are not equivalent. Whether they can both be used for one purpose (i.e. mutation detection) does not suggest that both can be equivalent for a different type of reaction producing different types of products.

CEL I used for mutation detection is not the invention. Oleykowski et al provides no suggestion that CEL I could be used to make sequence variants. Oleykowski et al provides no indication that CEL I is involved in DNA repair, the purpose of Arnold et al. Therefore, one lacks any suggestion to use CEL I for performing the claimed invention or even in the proposed combination of references.

The position stated in the rejection is that CEL I can be substituted for EndonucleaseVII in Birkenkamp et al. Even if this were done, one would still not have the present invention. At best one might imagine a better way for detecting mutations. This is not the present invention, which is a reaction to yield new DNA molecules with variant sequences from either parent DNA. Since no possible operable combination of the references suggests the claimed invention, this rejection should be withdrawn.

In view of the amendments and comments above, the rejections have been overcome. Reconsideration, withdrawal of the rejections and early indication of allowance are respectfully requested.

If needed, applicants petition for an extension of time under the provisions of 37 CFR 1.136(a) for sufficient time to accept this response. The commissioner hereby is authorized to charge payment of any fees under 37 CFR § 1.17, which may become due in connection with the instant application or credit any overpayment to Deposit Account No.500933.

Respectfully submitted,

Date: October 1, 2004

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